

## INTRACELLULAR BACTERIA IN THE GREEN SCALY PRASINOPHYTE *TETRASELMIS*

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ABSTRACT. — Ultrastructural investigation has shown the presence of intracellular bacteria in the green scaly flagellate *Tetraselmis*. The bacteria appear to be causing cellular damage. Three different types of relationships between algae and their intracellular bacteria are recognized and discussed.

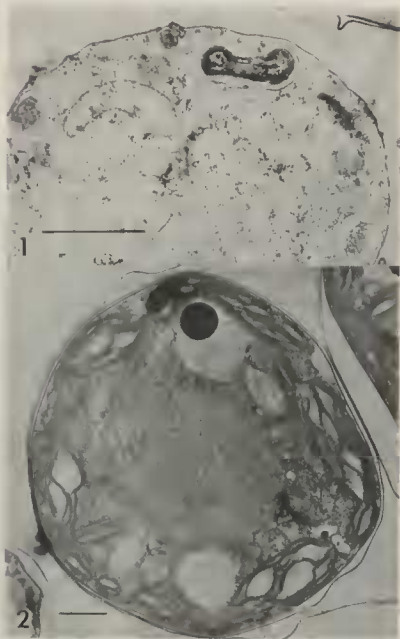
ZUSAMMENFASSUNG. — Elektronenmikroskopie Untersuchung hat bewiesen, das intrazelluläre Bakterien in den grünen, schuppenartigen Flagellat *Tetraselmis* existieren. Es scheint als ob die Bakterien in den Zellen der Alge Schaden verursacht haben. Drei verschiedene Verhältnisse zwischen den Algen und ihre intrazellulären Bakterien werden anerkannt und diskutiert.

### INTRODUCTION

Intracellular bacteria have been observed in a wide variety of algal species including representatives of the Cyanophyceae (WUJEK, 1979), Chloromonadophyceae (HEYWOOD, 1978), Chlorophyceae (MISHRA, 1969; BURR and WEST, 1970; KOCHERT and OLSON, 1970; TURNER and FRIEDMAN, 1974; LEE and KOCHERT, 1976; COLOMBO, 1978; DAWES and LOHR, 1978), Chrysophyceae (BELCHER, 1969; SWALE, 1969; HIBBERD, 1971; WUJEK, 1978, 1982), Dinophyceae (SILVA, 1959, 1962, 1963, 1967, 1978; GOLD and POLLINGHER, 1971; DODGE, 1973) and Xanthophyceae (OTT, 1979). In this paper we document the presence of bacteria in what appear to be degenerating cells of *Tetraselmis* c. f. *carteriiformis* Butcher. This is the first account of intracellular bacteria for a member of the class Prasinophyceae, the scaly green algal flagellates.

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### MATERIALS AND METHODS

A clone (J-7) of *T. c.f. carteriiformis*, was obtained from a single cell isolated by micropipette from a plankton sample collected (9 November, 1979) from Cockroach Bay, a shallow mangrove embayment confluent with Tampa Bay, Florida. It has been maintained in Provasolli's ES medium (PROVASOLLI, 1968) of a salinity of 23‰ at 20°C under  $3 \text{ nE}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$  of continuous cool-white fluorescent light. It is non axenic.

A sample from an exponential-phase culture of this clone was centrifuged gently and fixed in 2.5% glutaraldehyde in 0.07M  $\text{CaCl}_2$  plus 0.1M sodium cacodylate (pH 7.2) for 1 hour at room temperature. The fixed material was rinsed repeatedly in 0.1M sodium cacodylate, post-fixed in 1% osmium tetroxide for 1 hour at room temperature and again rinsed repeatedly in 0.1M sodium cacodylate. The cells were concentrated by mild centrifugation, embedded in 1.5% agar, dehydrated in a graded ethanol series and embedded in Spurr's epoxy resin (SPURR, 1969). Sections were made with a Sorval MT 2B ultramicrotome and stained with uranyl acetate and lead citrate. Sections representing more than 100 individual flagellates, were examined with either a Philips 200 or Hatachi H-500 electron microscope.

Live individuals of clone J-7 were examined with Nomarski interference microscopy and identified as *T. c.f. carteriiformis* using the key of BUTCHER (1959) which is based on characters observable with light microscopy (position of the eyespot, cell shape, etc.).

### OBSERVATIONS

Rod-shaped bacteria ( $2\text{-}3 \times 0.5\text{-}1\mu\text{m}$ ) were observed bounded by cytoplasmic vesicles in three individuals of *T. c.f. carteriiformis* (clone J-7). Bacteria found in the culture medium of this clone do not resemble these intracellular forms. The cells containing bacteria appear «unhealthy» from an ultrastructural standpoint, having degenerate membranes, etc. (fig. 1). Cells from the same culture fixed at the same time by the same procedure but not containing bacteria had cytological features typical for presumed healthy members of this genus (fig. 2 and STEWART et al., 1974; PEARSON & NORRIS, 1975; MELKONIAN, 1979). One of us (W.E.G.), while using light microscopy to examine live individuals of clone J-7, observed one pair of sister cells enclosed but their mother cell theca in which one sister was degenerate (apochlorotic) while the other was apparently healthy (pigmented normally).

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Fig. 1-2. — *Tetraselmis carteriiformis*. Fig. 1 : Section of a cell with an intracellular bacterium near the cell membrane. A nuclear area with what are presumably DNA fibrils can be observed within the bacterium. Fig. 2 : Section of an uninfected cell showing typical eukaryotic cellular organelles (from the same culture as fig. 1). Bar =  $1\mu\text{m}$ .

## DISCUSSION

The relationship between *T. c.f. carteritiformis* and its intracellular bacteria could be mutualistic or commensal as is apparently the case with certain euglenoid flagellates (LEEDALE, 1969), dinoflagellates (SILVA, 1978) and with *Chryso-sphaerella* (WUJEK, 1982). In these instances the bacteria reproduce intracellularly without apparently causing harm to their algal host. However, the «infected» individuals examined in this study appeared ultrastructurally degenerate when compared to «uninfected» cells fixed at the same time from the same culture and by the same procedure, suggesting a pathogenic relationship. The observation of an apochlorotic sister cell in the living culture is not inconsistent with this idea.

If the bacteria observed were pathogens, how do they infect a new cell after causing the death of a host. It is possible they live or subsist free in the medium between host infection cycles? Although the bacteria observed in the growth medium of the alga studied here were morphologically different from the intracellular forms, this does not prove they are different species. HEYWOOD (1978) suggested that the morphology of the intracellular bacteria of *Gonyostomum semem* might be affected by the internal environment of its host cell. This could also be the case with the bacteria we observed in *T. c.f. carteritiformis* in which case the possibility that extracellular bacteria had «infected» the cells we observed cannot be discounted.

Infection may begin with the attachment of the bacterium to the potential host. Subsequent endocytosis is one means by which the bacterium could gain entry the host cell. If this were to occur, the bacterium would like be enveloped by a membrane of host origin. This is apparently the case with the bacteria we found in *T. c.f. carteritiformis*, as well as those found in *Katodinium rotundatum* (DODGE, 1973) and *Volvox* (KOCHERT & OLSON, 1970; LEE & KOCHERT, 1976). In *Tetraselmis*, the cell membrane is protected by an external theca which should effectively prevent the endocytotic capture of a bacterium. However, the theca does not completely envelop the free-swimming stage and a potential infectious bacterium could gain access to the plasmalemma in the region of the flagellar pit. Although this would seem unlikely in an actively swimming cell, moribund individuals may be particularly susceptible to bacterial attachment as has been observed with the diatom *Skeletonema costatum* (KOGURE et al., 1982) and the red alga *Acrochaetium* sp. (LARPENT-GOURGAUD & DIDIER, 1981). Bacterial pathogens known to attack algae include *Bdellovibrio bacteriovorus*, which lyses *Phormidium luridum* and *Synechococcus* (BURNHAM & SUN, 1977), and *Bdellovibrio chlorellavorous* that attaches to and kills *Chorella vulgaris* (CODER & GOFF, 1979). These pathogens do not gain entry to the host cells as we have speculated above. However, they may well be exceptionally virulent and the need to be engulfed could be restricted to less virulent pathogens. Based on the literature at least three general types of relationships between intracellular bacteria and their respective algal hosts exist. In the first type, the bacteria are present in the cytoplasm but surrounded by a distinct membrane. This type is exemplified

by the bacteria found in *T. c.f. carteriiformis* (this study), *Volvox* (KOCHERT & OLSEN, 1970; LEE & KOCHERT, 1976) and *Katodinium rotundatum* (DODGE, 1973).

In a second type of relationship the intracellular bacteria are not bounded by any host membrane. These bacteria may be found in the cytoplasm as in *Gonyostomum semem* (HEYWOOD, 1978), *Vaucheria* (OTT, 1979) and *Pleurocapsa minor* (WUJEK, 1979); in the nucleus as found in certain euglenoid flagellates (LEEDALE, 1969) and *Gymnodinium splendens* and *Glenodinium foliaceum* (SILVA, 1978); or between the inner and outer membranes of the nuclear envelope as in *Chryso-sphaerella* (WUJEK, 1982). In the third type of relationship, the bacteria are found within the normal cytoplasmic vacuole, thus separate from the cytoplasm by the tonoplasts as has been found in the green algal coenocytes *Halimeda* and *Udotea* (COLOMBO, 1978), *Caulerpa* (MISHRA, 1969; DAWES & LOHR, 1978) and *Bryopsis* (BURR & WEST, 1970). The latter paper suggests that the bacteria are in the cytoplasm surrounded by an «electron transparent area». However, the micrographs of the vegetative cytoplasm (fig. 26, BURR & WEST, 1970) and gamete (fig. 41) of *Bryopsis* are similar to those of *Caulerpa* (DAWES & LOHR, 1978) and it appears the bacteria are actually in finger-like projections of the vacuole.

In summary, we have reported the occurrence of bacteria in cytologically degenerate cells of the scaly green algal flagellate *Tetraselmis c. f. carteriiformis*. This is the first report of intracellular bacteria in the Prasinophyceae. The relationship may involve pathogenesis, although further investigation is needed. There appear to be at least three general types of relationship between algae and their intracellular bacteria : 1) bacteria in cytoplasm and bounded by host membrane; 2) bacteria not bounded by host membrane and present in any of a number of places within the cells, and 3) bacteria within the normal cytoplasmic vacuole of the alga (as in coenocytic Chlorophyceae).

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